The litter vs. the individual offspring: Statistical Analysis and Biological Significance of Developmental Toxic Effects

Reinhard Meister

TFH Berlin

Objectives

This lecture aims at

- refreshing some basics of statistics
- explaining decomposition of data into signal and noise
- helping in understanding the data-structure emerging from litter based experiments
- presenting the amount of independent information provided by clustered data
- transferring concepts to quantal response case
- understanding litter based approaches to quantal response data

Decomposition of Data

- data $=$ signal $+$ noise
- $y = \mu + e$, components unobservable
- signal: μ assumed as fixed and real
  noise : e assumed as random $E(e) = 0, \quad Var(e) = \sigma^2$
- decomposition: $y_i = \widehat{\mu} + \widehat{e}_i, i = 1, \ldots, n$ by estimating components
- goal: reliable estimate of signal including measure of precision.
- precision of the mean, reached by a sample $y_1, \ldots, y_n$ of size n:

$$Var(\overline{y}) = \frac{\sigma^2}{n}$$

Variance of the mean decreases with sample size.

Statistical Basics in < 5 minutes

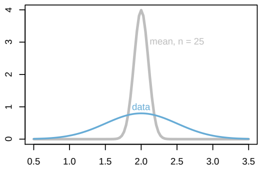
Data, Means, Confidence Intervals

example:1.94, 2.39, 0.29, 2.23, 2.56, 1.97, 2.23, 2.25, 2.57, 2.34, 2.41, 2.59, 2.20
1.70, 1.93, 2.26, 1.99, 2.37, 2.48, 1.62, 2.28, 2.84, 2.00, 2.43, 1.40

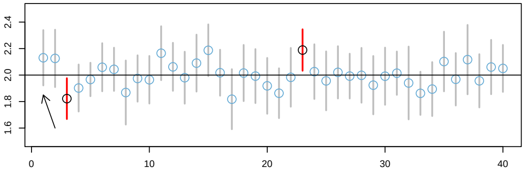
results : $n = 25, \quad \widehat{\mu} = \overline{y} = 2.13$ and $\widehat{\sigma} = 0.51$

95% CI: $\overline{y} \pm t_{0.975,n-1} \times \widehat{\sigma}/\sqrt{n} = (1.92, 2.34)$

Density of y and $\overline{y}$



40 simulated confidence intervals $\alpha = 0.05$



first interval is reported as example

Decomposition with Structured Noise

- data = signal + noise
- noise caused by litters b_i and by fetuses e_{ij}
  $i = 1, \ldots, I; \quad j = 1, \ldots, n_i$ for simplicity assume all n_i equal
- $yij = \mu + b_i + e_{ij}$, assumptions: b_i, e_{ij} independent,
  $Eb_i = Ee_{ij} = 0$, $Var(b_i) = \sigma_b^2$, $Var(e_{ij}) = \sigma^2$.
- data correlated within litters, no totally independent information

$$Corr(y_{ij}, y_{ik}) = \sigma_b^2/(\sigma_b^2 + \sigma^2) = \rho$$

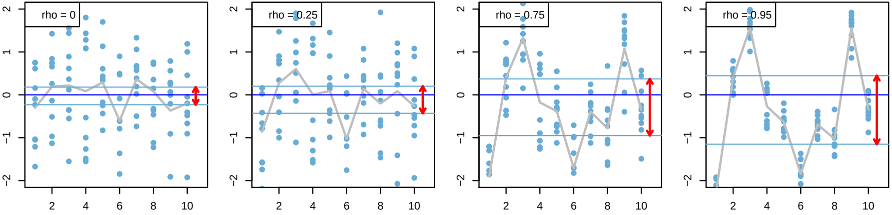
$$Var(\overline{y}_{..}) = \frac{1}{I}\left(\sigma_b^2 + \frac{\sigma^2}{n}\right) = \frac{1}{I \times n}\left(1 + n \times \frac{\rho}{1-\rho}\right)\sigma^2$$

Intra-litter correlation causes variance inflation of the mean

Example Litter Effect and Confidence Intervall

How intra-litter correlation changes inference

Data for 10 litters with 10 fetuses each are generated according to different correlations $\rho = 0, .25, .75, .95$, keeping the total variance of a single observation constant: $Var(y_{ij}) = 1$. Confidence intervals computed using estimates $\widehat{\sigma}_b^2$ and $\widehat{\sigma}^2$ from ANOVA decomposition.



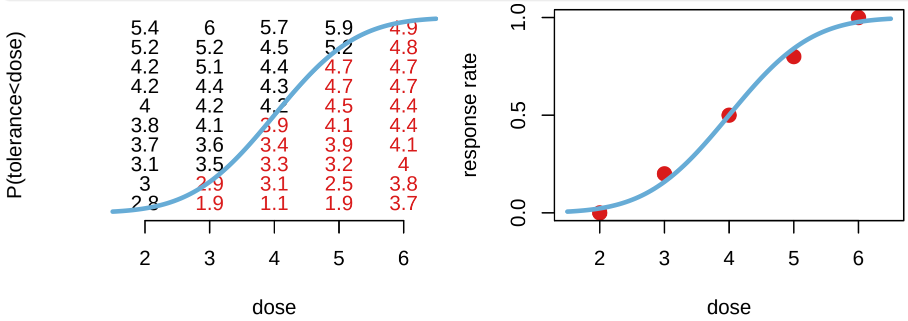
Confidence intervals get wider with increasing intra-litter correlation.

Tolerance and Quantal Data

Distribution of individual tolerances determines response probabilities

Hypothetical tolerances for 50 individuals randomly assigned to 5 dose groups.

Fit of quantal response model: Maximum Likelihood for binomial data.

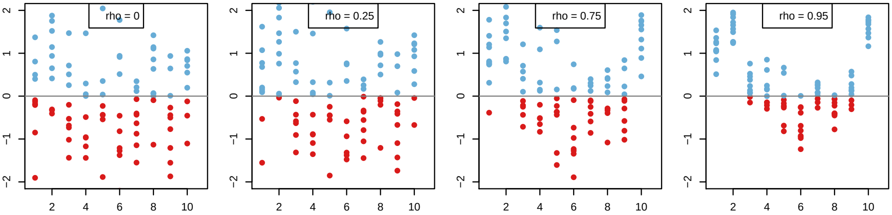


Quantal response allows indirect estimation of tolerance distribution

Litter Effects for Tolerances

How intra-litter correlation changes pattern of reactions

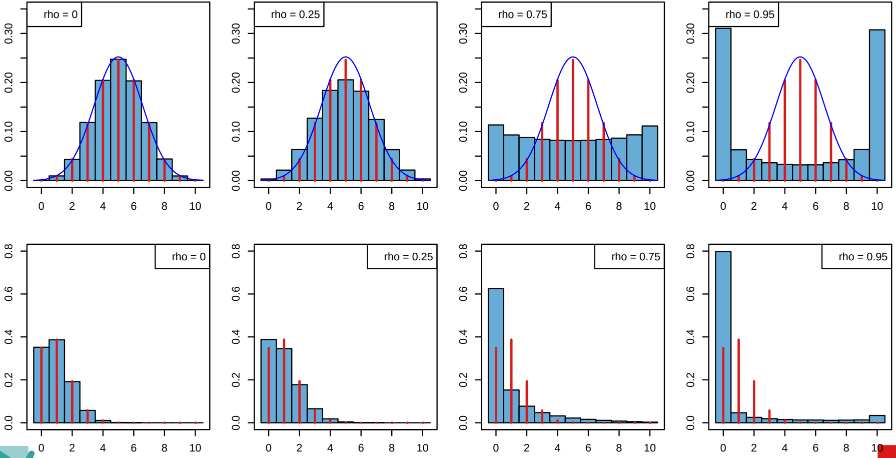
Tolerances with litter effects, 10 fetuses for 10 litters. Increasing intra-litter correlation.



Litter effects increase dissimilarities of response rates between litters

Example Litter Effect and Quantal Data

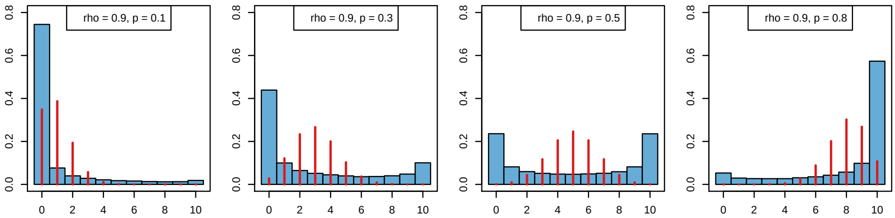
How intra-litter correlation changes distribution $p = 0.5, 0.1$



Intra-litter correlation results in overdispersed number of reactions per litter

Example Litter Effect and Quantal Data

How response probability changes distribution

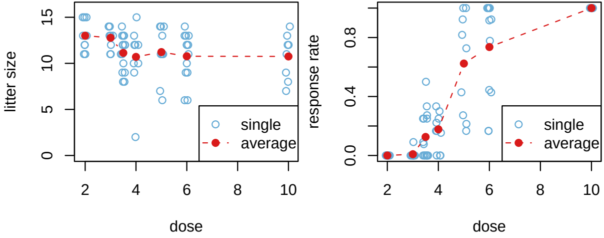


probability p	0.1	0.3	0.5	0.8
mean	1	3	5	8
variance	0.52	1.32	1.60	0.98
binomial variance	0.09	0.21	0.25	0.16
over-dispersion	5.73	6.30	6.41	6.11

Intra-litter correlations induces nearly constant overdispersion

Raw data from Platzeck et al

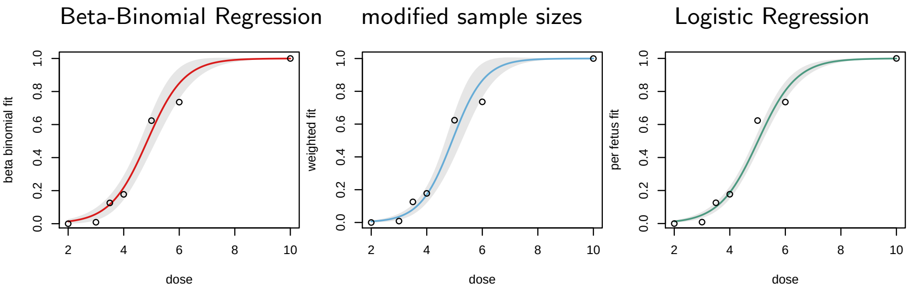
dose	reactions/litter size	$\sum r_i / \sum n_i$	litter effect
2	0/11 0/11 0/12 0/12 0/13 0/13 0/15 0/15 0/15	0	
3	0/11 1/11 0/12 0/13 0/13 0/13 0/14 0/14 0/14	0.009	–
3.5	0/8 2/8 0/9 3/9 5/10 1/11 3/11 0/12 0/12 3/12 3/12 0/13 0/13 1/13 0/14	0.13	**
4	0/2 2/9 0/10 3/10 0/12 2/12 2/12 3/12 2/13 5/15	0.18	–
5	1/6 7/7 3/11 8/11 9/11 12/13 3/14 6/14 14/14	0.62	**
6	6/6 6/6 4/9 7/9 10/10 11/11 2/12 2/12 11/12 12/13 13/13 13/13 6/14	0.74	**
10	7/7 8/8 9/9 11/11 12/12 12/12 13/13 14/14	1	



Results: slightly decreasing litter size, increase in response rate

MNU Cleft palate induced by MNU

How different treatment of litter-effect changes fit

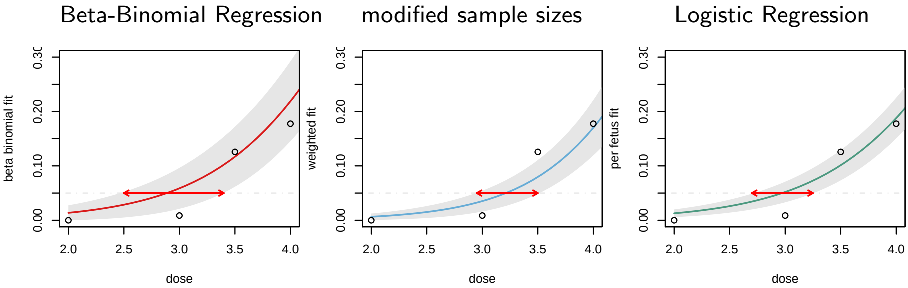


litter effects cause weighted fit to data

ignoring litter effects gives too narrow confidence bands

MNU Cleft palate induced by MNU

How different treatment of litter-effect changes benchmarks



Selected method influences value **and** width of confidence interval
beta binomial fit recommended

Conclusions

Rôle of litter-effects for data analysis in teratology

Quantitative data

- Precision: Variance of the mean decreases with sample size.
- Litters: Intra-litter correlation causes variance inflation of the mean.
- Confidence intervals get wider with increasing intra-litter correlation.

Qualitative data – quantal reponse

- Quantal response allows indirect estimation of tolerance distribution.
- Litter effects increase dissimilarities of response rates between litters.
- Intra-litter correlation results in overdispersed number of reactions per litter.
- Intra-litter correlations induces nearly constant overdispersion.
- Litter effects cause weighted fit to data.
- Selected method influences value and width of confidence interval,beta binomial regression with constant overdispersion recommended.

References

Zhu Y, Wang T, Jelsovsky JZ. Bootstrap estimation of benchmark doses and confidence limits with clustered quantal data. Risk Anal. 2007 Apr;27(2):447-65.

Pang Z, Kuk AY. Test of marginal compatibility and smoothing methods for exchangeable binary data with unequal cluster sizes. Biometrics. 2007 Mar;63(1):218-27.

Pang Z, Kuk AY. A shared response model for clustered binary data in developmental toxicity studies. Biometrics. 2005 Dec;61(4):1076-84.

Hunt D, Rai SN. Testing threshold and hormesis in a random effects dose-response model applied to developmental toxicity data. Biom J. 2005 Jun;47(3):319-28.

Regan MM, Catalano PJ. Regression models and risk estimation for mixed discrete and continuous outcomes in developmental toxicology. Risk Anal. 2000 Jun;20(3):363-76.

Regan MM, Catalano PJ. Likelihood models for clustered binary and continuous outcomes: application to developmental toxicology. Biometrics. 1999 Sep;55(3):760-8.

Ryan L, Molenberghs G. Statistical methods for developmental toxicity. Analysis of clustered multivariate binary data. Ann N Y Acad Sci. 1999;895:196-211.

Morel JG, Neerchal NK. Clustered binary logistic regression in teratology data using a finite mixture distribution. Stat Med. 1997 Dec 30;16(24):2843-53.

References

Schuster C, Meister R. Benchmarks for Quantitative Endpoints: An Approach Accounting for Litter Effects. Communications in Statistics. 1996; 25(12):3085-99
Fung KY, Krewski D, Rao JN, Scott AJ. Tests for trend in developmental toxicity experiments with correlated binary data. Risk Anal. 1994 Aug;14(4):639-48.
Ryan L. Using historical controls in the analysis of developmental toxicity data. Biometrics. 1993 Dec;49(4):1126-35.
Carr GJ, Portier CJ. An evaluation of some methods for fitting dose-response models to quantal-response developmental toxicology data. Biometrics. 1993 Sep;49(3):779-91.
Ryan L. The use of generalized estimating equations for risk assessment in developmental toxicity. Risk Anal. 1992 Sep;12(3):439-47. Review.
Ryan L. Quantitative risk assessment for developmental toxicity. Biometrics. 1992 Mar;48(1):163-74.
Piegorsch WW, Haseman JK. Statistical methods for analyzing developmental toxicity data. Teratog Carcinog Mutagen. 1991;11(3):115-33. Review
Williams DA. Estimation bias using the beta-binomial distribution in teratology. Biometrics. 1988 Mar;44(1):305-9.

References

Nelson CJ, Felton RP, Kimmel CA, Buelke-Sam J, Adams J. Collaborative Behavioral Teratology Study: statistical approach. Neurobehav Toxicol Teratol. 1985 Nov-Dec;7(6):587-90.

Shirley EA, Hickling R. An evaluation of some statistical methods for analysing numbers of abnormalities found amongst litters in teratology studies. Biometrics. 1981 Dec;37(4):819-29.

Crowder MJ. Beta-binomial Anova for proportions. Applied Statistics. 1978;27: 34-37.

Kupper LL, Haseman JK. The use of a correlated binomial model for the analysis of certain toxicological experiments. Biometrics. 1978 Mar;34(1):69-76.

Holson JF, Scott WJ, Gaylor DW, Wilson JG. Reduced interlitter variability in rats resulting from a restricted mating period, and reassessment of the litter effect". Teratology. 1976 Oct;14(2):135-41.

Haseman JK, Hogan MD. Selection of the experimental unit in teratology studies. Teratology. 1975 Oct;12(2):165-71.

Healy MRC. Animal Litters as Experimental Units. J. Royal Stat. Soc. (Series C), Applied Statistics. 1972;21(2): 155-159.